＜Instruction Manual＞

House dust mite allergen (Der 2) ELISA Kit

Intended Use

The Nichinichi Der 2 ELISA kit is used for the quantitative measurement of a major allergen of dust mite, Der 2.

Highlights

- Total Assay Time: 5 hours 20 mins
- Assay Range: 100–6400 pg/mL
- Containing all reagents required for assay
- High specificity and low variability

Reagents Provided

1）	Antibody coated plate	8wells × 12
2）	ELISA diluent	50mL × 1
3）	Wash buffer (20x)	50mL × 1
4）	Stop solution	20mL × 1
5）	Substrate reagent A	7mL × 1
6）	Substrate reagent B	7mL × 1
7）	Standard Der 2 (64 ng/mL)	0.3mL × 1
8）	Detection antibody	100μL × 1
9）	Streptavidin-labeled HRP	15μL × 1
10）	Instruction manual	1

Reagents preparation and assay procedure

1. Reagents Preparation

（1） Standards

The calibrator is set at a concentration of 64 ng/ml (white-cap tube). Perform serial dilutions with ELISA diluent to make standards of 100, 200, 400, 800, 1600, 3200 and 6400 pg/ml. The 6400 pg/ml standard serves as the standard with the highest concentration. The ELISA diluent serves as the "zero-standard" (0 pg/ml). Der 2 standards need to be prepared immediately before use.

Example for duplicate

Final conc. (pg/mL)	6400	3200	1600	800	400	200	100
Standard (μL) 64ng/ml:	60	300	300	300	300	300	300
ELISA diluent (μL)	540	300	300	300	300	300	300

（2） Wash buffer

Prepare 1x wash buffer by adding concentrated detergent solution (50ml of 20x) to 950 ml of distilled

or deionized water. Mix well and store the wash buffer at room temperature before use. Diluted wash buffer (1x) may be used for next assay when stored at 2-8°C. Do not use if contamination is suspected.

（3） Detection antibody

Dilute to 1: 650 with ELISA diluent immediately before use.

（4） Streptavidin-labeled HRP

Dilute to 1: 100,000 with ELISA diluent immediately before use (e.g. Add 10 μL of Streptavidin-labeled HRP solution to 1 mL of ELISA diluent (100 hold dilution). Then, take 10 μL of this solution and add to 10 mL of ELISA diluent).

（5） Substrate solution

Mix equal volumes of Substrate regent A and Substrate regent B immediately before use. Avoid exposure of substrate to direct light. Discard any unused mixed substrate solution.

2. Assay Procedure

（1） Remove any unused test stripes/wells from well holder, and return to sealed pouch for 2-8°C storage.

（2） Pipette 100 μl of each standard and sample into appropriate wells.

（3） After mixing, incubate the plate stationary for 2 hours at 37°C.

（4） Decant or aspirate the contents of all the wells. Wash each well three times with at least 250 μl of freshly prepared wash buffer (see *Reagent Preparation*, Step 2) by decantation or aspiration. After the last wash, blot the plate on absorbent paper towels to remove any residual buffer. Complete removal of the buffer is required for optimal results.

（5） Pipette 100 μl of the freshly prepared detection antibody (see *Reagent Preparation*, Step 3) to each well.

（6） After mixing, incubate the plate stationary for 2 hours at 37°C.

（7） Decant or aspirate the contents of all the wells. Wash each well three times with at least 250 μl of freshly prepared wash buffer (see *Reagent Preparation*, Step 2) by decantation or aspiration. After the last wash, blot the plate on absorbent paper towels to remove any residual buffer. Complete removal of the buffer is required for optimal results.

（8） Pipette 100 μl of the freshly prepared streptavidin-labeled HRP (see *Reagent Preparation*, Step 4) to each well. Incubate the plate stationary for 1 hour at 37°C.

（9） Decant or aspirate the contents of all the wells. Wash each well six times, and blot the wells as in *Assay Procedure* Steps 7.

（10） Pipette 100 μl of the substrate solution (see *Reagent Preparation*, Step 5) into each well.

（11） Incubate in the dark at 37°C for appropriate minutes (about 20 minutes). Add 100 μl of the stop solution to each well for stopped reaction.

（12） Read the absorbance at 450 nm within 15 minutes from adding the stop solution.

Der 2

Flow Chart

Antibody coated plate

↓

100 μl of standards and samples

↓

Mix gently and incubate the plate stationary for 2 hours at 37°C

Wash three times （250-300μL/well ×3）

↓

100 μl of freshly prepared detection antibody

↓

Mix gently and incubate the plate stationary for 2 hours at 37°C

Wash three times （250-300μL/well ×3）

↓

100 μl of freshly prepared streptavidin-labeled HRP

↓

Incubate the plate stationary for 1 hour at 37°C

Wash six times （250-300μL/well ×6）

↓

100 μl of freshly prepared substrate solution

↓

Incubate the plate at 37°C for 20 minutes （Incubation time may be optimized depending on the performance of spectrometer used）.

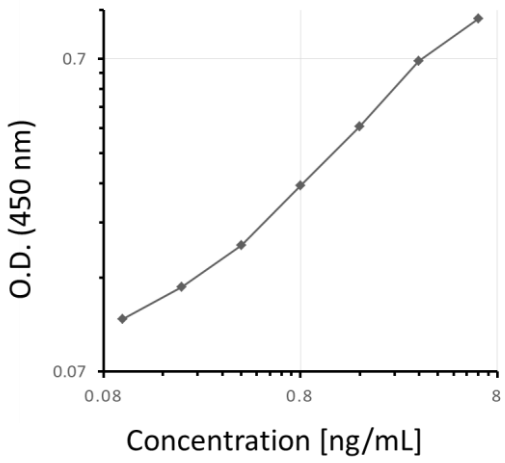
↓

100 μl of stop solution

↓ Mix

Abs at 450nm

Sample Curve



（1） The value of absorbance at 450 nm may be variable depending on the experimental conditions during color development.
（2） The assay range is 100–6400 pg/mL.
（3） If a curve fitting program is available, the 3rd order polynominal fitting is recommended.

Storage and Stability

（1） Store the unopened kit at 2–8°C (Do not freeze).
（2） The regents are stable for 6 months since the manufacture date stated on the box.
（3） Once opened, use as soon as possible.

Precautions and Limitations of the Procedure

（1） Store unused strips/wells at 2–8°C in the tightly sealed pouch.
（2） Allow all reagents to reach room temperature before use.
（3） Avoid contact of skin, eyes, or clothing with reagents since some of them are toxic. In case of contact, flush immediately with large amount of water.
（4） The components of this kit should not be used for any other purposes.
（5） The components of this kit are intended for use as an integral unit. The components and reagents of different kits should not be mixed with those of this kit.
（6） This test kit is designed for research use only and not for diagnostic or therapeutic purposes.

※ All claims must be made within 7 days after the expiry date. Nichinichi Pharmaceutical Co., Ltd. will replace any defective products with the same number of new products. Nichinichi Pharmaceutical Co., Ltd. assumes no responsibility for any damages or losses (including direct, indirect, special, consequential, or other) which may occur with the use of this kit.

Manufacturer
For inquiries, please contact:
Nichinichi Pharmaceutical Co., Ltd.
239-1 Tominaga, Iga, Mie 518-1417, Japan
Fax: +81-595-48-0209
www.nichinichi-phar.co.jp
elisa@nichinichi-phar.co.jp

(6. 6. 2022)